

THE EXAMINATION OF CROSS-FOSTERING WITH ICR MICE
AS A MODEL FOR FOSTER CARE

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ABSTRACT

Foster care youth face significant challenges and are at greater health risk than their non-fostered counterparts (Kools et al., 2009). The current research seeks to investigate behavioral impacts of cross fostering (CF) and repeated cross fostering (RCF) in early life. This design can then be implicative of what foster children experience when they are moved from home to home. Nine timed pregnant ICR mice were randomly assigned to the control, CF, or RCF group. RCF pups were placed with a foster mother on PND 1 and then moved to a second foster mom on PND 11. CF pups moved to a foster mom on PND 1. Maternal behavior was noted with cross fostering. At PND 21 the pups began behavioral testing (OFT, EZM, MWM, and ADL). RCF and CF mice showed more anxiety compared to the controls. Early life stress plays a large role in behavior and development.

Keywords: cross foster, repeated cross foster, early life stress

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INTRODUCTION

According to the United States (U.S.) Department of Health and Humans Services (HHS) (2018), since 2012 the number of children entering the foster care system has been increasing by around 10,000 every year in the United States. The experience of foster care places many burdens and difficulties on the lives of these children. Foster care youth are at high risk for disruptions in mental development and have increased rates of mental disorders that extend into adulthood (Leve et al., 2013). Research indicates that those who have experienced foster care have difficulty securing employment, have less education, and are at a greater risk of experiencing homelessness (Zlotnick et al., 2012). Moreover, children in foster care also face greater challenges and are the most vulnerable with concerns to their health when compared to any other children in the United States (Kools et al., 2009).

There are close to half a million children in the foster care system in the United States in any given year (U.S. Department of HHS, 2019). In fiscal year (FY) 2016, child protective services investigated allegations of neglect or abuse involving some 3.5 million children (U.S. House of Representatives, 2018). In FY2018, of the children entering the foster care system, neglect and drug abuse by one or both parents were the top circumstances surrounding the child's removal (U.S. Department of HHS, 2019).

The foster care system is in place as a way for state authorities to protect children by intervening in the family unit (Rymph, 2018). Children are removed from homes of abuse and neglect under the authority of a local court (Dannerbeck, 2007; Rymph, 2018). Foster care includes the placement of children into adoption homes and orphanages, which some

may see as distinct from foster care when indeed it is not (Rymph, 2018). In 1980, the federal government passed a law to try to reduce and end the changes in placement of children and encourage permanency even if it was not with the biological parents (Rosenfeld et al., 1997). Although this was a step in the right direction, the effect did not last, as can be seen with the continuous problem of placement instability in the current decade.

Most of the foster youth in FY2018 were placed with foster families, as compared to group homes, institutions, or adoption (U.S. Department of HHS, 2019). One-third of the children in foster care during FY2018 were under the age of four (U.S. Department of HHS, 2019). Due to these concerns and statistics, the goal of this project was to focus on the importance and impact early life stress can impose on young children, and how this experience early in life can have many consequences.

Few studies have investigated this type of stressful experience in animal models. However foster mothers are often used in research labs to save and increase the survival of mouse pups for ongoing experiments (Lerch et al., 2014). Luchetti and colleagues (2015) investigated early handling and repeated cross fostering as a model of early life stress, and how these early life events can have a crucial role in the phenotype of the individual. They found that animals exposed to repeated cross fostering showed enhanced sensitivity and reduced emotionality (Luchetti et al., 2015). One study conducted by Barbazanges and colleagues (1996) examined whether or not having an early adoption versus a late adoption would have long-term effects on male offspring specifically. This research study was investigating adoptions several hours after birth versus five or twelve days after birth and found that the time of the adoption induced different and even opposite effects on the mouse

pups in adulthood (Barbazanges et al., 1996). It was found that the both early and later separations led to a higher stress response through corticosterone secretion; it was also found that later adoptions were linked to decreased memory (Barbazanges et al., 1996).

Studies using animals have shown that early environment manipulation can impact neurobiology and behavior of the animal (Sengi et al., 2018). However, most of these studies have focused on the long-term effects on the offspring. In most species the perinatal and postnatal periods are the most sensitive periods in life (Sengi, 2018). Sengi and colleagues (2018) found that the early environment plays quite an influential role in the developmental trajectories of the animals through modulating the stress response, imprinting changes in neurochemical and neuroendocrine reactions, and inducing vulnerabilities to psychopathologies (Sengi et al., 2018).

Of the studies that have examined this type of early life stress, there are limitations. A majority of the studies looking at this type of manipulation early in life have only assessed male offspring (Barbazanges et al., 1996; Luchetti et al., 2015; Sengi et al., 2018). No studies investigating this topic have looked at non-cognitive behaviors, including activities of daily living, such as burrowing and nesting. Although, Lerch and colleagues (2014), investigated cross fostering and nesting among other variables, no studies have investigated repeated cross fostering (RCF), cross fostering (CF), and activities of daily living.

Early Life Stress

Children in foster care experience forms of early life stress which lead to long-term behavioral and neural development issues; these have been seen in both humans and non-human animals (Goodwill et al., 2018). Examples of these forms of early life stress can

include childhood physical, emotional, and/or sexual abuse, neglect, parental separation, and childhood trauma (De Bellis & Zisk 2014; Kaufman et al., 2000).

A majority of children with a history within the foster care system have experienced early life stress through neglect, abuse, caregiver disruptions, or a combination of these (Cowell, 2015). When compared to other children, these experiences impact foster children both during foster care and afterwards in the forms of physical and mental health, educational disruptions, and neurological and developmental setbacks, to name a few (Burskas, 2008; Fisher et al., 2011; Steenbakkens et al., 2018). Along with these types of early life stress, foster youth also often experience the loss an attachment figure.

Children who experience loss of a parent or caretaker will exhibit distress even if that figure is replaced by a capable and compassionate caretaker (Bowlby, 1982). This separation of parent and child is a severely threatening experience for the child, irrespective of the quality of the care or the quality of experiences the child has had with the parent (Folman, 1998). Separation is often distressing and anxiety-provoking which in turn can manifest in behavioral problems (McWey et al., 2010). This disruption of the child-parent relationship can cause the child to feel like he or she is being disloyal and betraying his or her parents (McWey et al., 2010).

Children in the foster care system can be removed from their biological parents for a large variety of reasons, and this removal can have major impacts on both the mental health of the child and on his or her development (Kools et al., 2009; Pecora et al., 2009; Steenbakkens et al., 2018; Zlotnick et al., 2012). Reasons for removal can include but are not limited to: substance abuse by one or both parents, mental illness of one or both parents,

safety concerns for the child (violence, abuse and/or neglect), lack of stable living arrangements, parents' lack of resources, homelessness, and/or parental arrest or incarceration (Dannerbeck, 2005; Hayward & DePanfilis, 2007; Pelton, 2007). Most children in foster care experience the loss of an important figure; to compound that stress, they also commonly experience moving into a new environment and too often have to cope with placement instability.

Placement Instability

Placement instability has been a concern of social workers for children in the foster care system for many years (Smith et al., 2001). Placement changes are a common occurrence for youth in foster care (Connell et al., 2006). Placement instability and the number of placement changes experienced in foster care may exacerbate and intensify the negative prospects of foster youth (Havlicek, 2010; Smith, et al., 2001). Children can experience a sense of rejections and impermanence with each change in placement; this can lead to decreases in the ability for the child to form emotional ties with his or her caregivers (Webster et al., 2000). Although findings are variable from study to study, the experience of placement instability for foster youth is commonplace (Collazo, 2013). In one study, Connell and colleagues (2006) found foster youth in Rhode Island had an average of three placements from 1998 to 2002, with the highest being 37 different placements. Disruptions of placements have been reported as high as 57% in the first year of placement with percentages increasing with the more time spent in foster care (Smith et al., 2001). Webster and colleagues (2000) found 50% of the foster youth in California had experienced three or more placement changes in the matter of eight years. As many as two-thirds of foster placements

are disrupted within the first two years, and half of new foster placements are disrupted within the first six months (Fisher et al. 2011).

Placement changes are associated with many negative psychological and health outcomes including compromised developmental trajectories which can lead to attachment difficulties, behavior problems, and juvenile delinquency (Connell, et al., 2006). Even in the best cases, children still experience a disruption in their continuity of care which can lead to reciprocal rejection and alienation (Webster et al., 2000).

Correlates of placement instability within the foster care system have been investigated. Reasons for removal have been found to be a risk factor for placement instability (Webster et al., 2000). Children who were removed for reason of maltreatment, (i.e. physical or sexual abuse) were found to be less likely to experience placement changes when compared to children who have been removed due to neglect (Webster et al., 2000). Older children and those with prior maltreatment have been found to be more likely to suffer placement instability (Connell et al., 2006). Children in kinship care as compared to non-kinship care experience fewer placement moves (Webster et al., 2000). The younger a child is when entering the system, the more likely he or she is to experience placement instability (Webster et al., 2000).

Too many moves can also disrupt educational progress, with foster children being one of the most educationally vulnerable populations in schools (Allen & Vacca, 2010; Webster et al., 2000; Zetlin & Weinberg, 2004). The number of placements a child experiences impacts academic performance, health care needs, and leads to developmental and behavioral problems (Allen & Vacca, 2010). Through the multiple moves these children face, personal

histories are often misunderstood or go unnoticed even with the impact it has on school performance (Zetlin & Weinberg, 2004). Foster youth often miss many days of school while in transition from home to home, and then once in a new home have to face the challenges of being in a new school (Bruskas, 2008). Schools are often not equipped to address the significant academic, behavioral, and emotional problems experienced by foster youth (Zetlin & Weinberg, 2004). With these difficulties, foster children experience lower grades, grade retention, and/or placement in special education, on top of foster youth being twice as likely to dropout before completion of high school (Zetlin & Weinberg, 2004). Constant moving can also impact credit transfer from school to school further adding to the challenges faced by foster youth in the education system (Bruskas, 2008).

Along with placement instability and changes in placement, these children are often placed in unstable environments (Havlicek, 2010). They have experienced a disruption in their supportive networks and relationships with extended family and other adults (Havlicek, 2010). Childhood maltreatment including neglect and abuse represents a failure of the child's environment (Cowell et al., 2015). Placement instability and traumatic experiences already create an unstable environment for foster youth (Bruskas, 2008; Havlicek, 2010). This setting is further amplified by having low parental care, few opportunities for social or cognitive growth, and/or problems with school (Connell et al., 2006; Steenbakketers et al., 2018). These unstable environments may exacerbate the negative outlook on the future of foster youth (Havlicek, 2010).

Brain Development and Trauma

Children in foster care often experience neglect, and as stated before, this is one of

the most common reasons for placement changes and children entering the system. This neglect, especially if long-term, can have permanent long-lasting impacts during the development of the susceptible brains of foster youth. Therefore, this experience can lead to changes in brain morphology and further lead to behavioral and psychological differences and difficulties that are otherwise not seen in children without this experience of neglect.

Children who experience this type of neglect, abuse and/or chaos as they are growing up are not able to have the fundamental developmental experiences in order to self-regulate, communicate, relate, and think (Perry, 2006). They are often under socialized, and have more emotional, behavioral, cognitive, and physical health problems (Kools et al., 2009; Perry, 2006). Along with many mental health and developmental disparities, children who have experienced foster care are also inclined to have higher incidences of physical health disparities (Zlotnik et al., 2012).

At earlier stages of development, the brain is more malleable, especially in the first year of life when the greatest amount of growth and change is happening (Cowell et al., 2015). Studies have shown that stress in early life can induce long term changes in neurotransmitter systems and brain structures such as the HPA axis, prefrontal cortex, and the hippocampus (Gunnar & Quevedo, 2008; Herpfer et al., 2012; Kaufman et al., 2000; Sarabdjitsingh et al., 2017). The neurobiological changes that come from the result of early life adversity that foster youth have been shown to experience results in a vulnerability of this population to developmental and psychiatric disorders (Kaufman et al., 2000).

When responding to stress, the human body has mechanisms in place; one of them includes the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is highly responsive

to stress and helps the body respond to the outside environment by regulating its response to stressful events and releasing hormones such as corticotrophin releasing hormone and cortisol (Fisher et al., 2010; Gunnar & Quevedo, 2008). During the first year of life the HPA axis in humans becomes progressively less responsive (Gunnar & Quevedo, 2008). There is a strong social regulation and parental buffering of the HPA axis during the first year of life (Gunnar & Quevedo, 2008). Early life stressors can impact the HPA system and its response to stress leaving children with a heightened vulnerability to stressors (Gunnar & Quevedo, 2008). Chronic stress and severe early life stress, often experienced by children in foster care, has been shown to blunt the diurnal rhythm of the HPA axis, lowering the production of cortisol in the morning and leading to behavioral and emotional problems in childhood and immunosuppression (Fisher et al., 2010). One biological theory posits that chronic stress evokes hyperactivity in the HPA axis leading to hypercortisolemia and atrophy of the hippocampus, an important structure for learning and memory (Richards & Wadsworth, 2004).

Brain plasticity is also highly sensitive to stress (Herpfer et al., 2012). Early life adversity is strongly associated with lower cognitive ability and lower cognitive growth in both childhood and adolescence, including in measures of memory (Richards & Wadsworth, 2004). Early life stress has been shown to affect memory in a number of different ways, with effects taking place immediately and continuing into adulthood (Morris et al., 2016). These experiences impact memory, executive function and attention, as well as learning (Vasilevski & Tucker, 2015). One of the largest impacts early life stress has on memory is specifically with working memory, although it has been shown to impact other types of memory

including short-term memory, long-term memory, as well as episodic memory (Masson et al., 2015; Morris et al., 2016). Working memory has been shown to be worse in children who have experienced early life stress, the earlier this experience the greater the impact, when compared to children of the same age who have not, as well as in maltreated adolescents when compared to unaffected controls (Cowell et al., 2015; Vasilevski & Tucker, 2015). Emotional and physical abuse in childhood has been shown to disrupt autobiographical memory, with children reporting less specific memories of their childhoods (Morris et al., 2016).

Maltreated children also experience negative impacts on other regions of the brain including but not limited to the prefrontal cortex, the anterior cingulate cortex, and the amygdala (Cowell et al., 2015). Early life stress has been shown to reduce the volume of the prefrontal cortex, the orbitofrontal cortex, the hippocampus, as well as white matter volume (Herpfer et al., 2012; Sarabdjitsingh et al., 2017). These disparities show deficits in inhibition, reasoning, planning, problem-solving, self-control, and other cognitive abilities (Cowell et al., 2015). Studies have shown similar deficits and developmental problems from exposure to experiences of early life stress in non-human animal models (Goodwill et al. 2018; Mehta & Schmeuss, 2011; Sarabdjitsingh et al., 2017).

Animals Models

Structural and neurobiological changes in the brain as a result of early life stress have also been shown to have impacts on behavior and the brain in animal models. Many animal models have examined early life stress and the impacts it may have on both behavior and biology in animals. Early life stress in animals has been modeled by investigating maternal

separation, maternal deprivation, limited bedding, cross fostering, and/or repeated cross fostering, (Goodwill et al., 2018; Lu et al., 2009; Luchetti et al., 2015; Mehta & Schmauss, 2011; Sarabdjitsingh et al., 2017). Early life stress has been shown to lead to depression-like behavior (Goodwill et al. 2018), structural changes in the brain (Sarabdjitsingh et al., 2017), and cognitive deficits (Mehta & Schmeuss, 2011). More specifically, cross fostering and repeated cross fostering in rodents have been shown to lead to long lasting emotional changes, including mood and anxiety disorders (Lerch et al., 2014; Lu et al., 2009; Luchetti et al., 2015). Early life stress can play a large role in programming phenotype and traumatic experiences can lead to neurological disorders later in life (Luchetti et al., 2015).

Animals who were exposed to prenatal and postnatal stress paradigms have been shown to have long-term neurobiological changes (Kaufman et al., 2000). Models of early life stress such as maternal deprivation have been shown in animal models to induce changes in neurobiology including increases in corticotropin releasing hormone (CRH), norepinephrine, adrenocorticotropin, as well as reduction in GABA and HPA activity (Gunnar & Quevedo, 2008; Kaufman et al., 2000). Early life stress has been shown to impact the size of the hippocampus in rodents and monkeys, with animals exposed to early life stress having smaller hippocampal volumes and less neurogenesis when compared to animals who have not had this exposure (Kaufman et al., 2000). Early life stress has also been associated with compromised function in the prefrontal cortex, similar to deficits seen in humans experiencing stressful events (Cowell et al., 2015).

ICR (CD-1) Mouse Strain

ICR mice have been found to have good characteristics for maternal care (Zivkovic et

al., 2016). Martin-Sanchez and colleagues (2015) found ICR mice to have high motivation towards pups, as assessed by recording pup retrieval times. They also found that lactating females had higher motivation when compared to non-lactating female mice (Martin-Sanchez et al., 2015). Neonatal cannibalism has also been found to be very low in ICR mice (Zivkovic et al., 2016). One study found ICR mother mice to have a preference for their own pups over pups from another mother, with a finding of ICR mice having a mutual recognition between mother and infant (Mogi et al., 2017). ICR mothers have been found to spend the same amount of time in parental behavior and providing maternal care regardless of if the father was present or not (Wright & Brown, 2000).

One of the most important influences in early life is between the primary caregiver and the offspring (Ladd et al., 2000; Martin-Sanchez et al., 2015). Maternal care is a tremendous factor in the healthy development of the infant (Martin-Sanchez et al., 2015). The pup-mother bond is one of the earliest and amongst the strongest of social attachments that are formed by most mammals (Sengi et al., 2018). In animal models, the mother is nearly exclusively the main environmental element with which the pup interacts, and therefore plays a critical role in its development (Sengi et al., 2018). Both cross-fostering and repeated cross-fostering interfere with this element.

Cross-Fostering

Cross-fostering has been shown to induce physiological and behavioral alterations in rodent models (Bartolomucci et al., 2004; Lerch et al., 2014; Lu et al., 2009; Santangeli et al., 2016). Previous research has shown cross-fostering to influence weight, emotionality, anxiety, corticosterone levels, as well as other behavioral and biological measures in pups

(Bartolomucci et al., 2004; Lerch et al., 2014; Lu et al., 2009; Santangeli et al., 2016; Siviyy, 2017). There is a dearth of research exploring cross-fostering and the impact it may have overtime. Previous studies examining cross-fostering have been focused on the mother and how maternal care affects the pups or have only investigated biological changes of the pups (Francis et al., 1999; Liu et al., 2000; Priebe et al., 2005; Santangeli et al., 2016). In the current study, models of cross-fostering are being investigated for the impacts it can have on development and behavior of the pups specifically.

Repeated Cross-Fostering

Repeated cross fostering has been shown to enhance sensitivity to negative events in adult life in mice (Sengi et al., 2018). RCF has been used to model human early environmental instability through postnatal manipulation (Luchetti et al., 2015). RCF places an impact on the infant-mother attachment bond by either disrupting or preventing it (Luchetti et al., 2015). This disruption predisposes the offspring to separation anxiety without inducing caregiver neglect (D'Amato et al., 2011). Variation in maternal care can lead to different developmental pathways in offspring (Curley & Champagne, 2016). Very little research has been done investigating repeated cross-fostering and its impact on behavior. By including repeated cross-fostering in this study, it can be investigated how the rearing environment and maternal care impact the behavior and physiology of the animals.

There is a lack of prior research on this topic. Repeated cross fostering has not been studied in depth and the behavioral and especially non-cognitive measures have not been investigated in pups under these manipulations.

Purpose/Hypothesis

The present study pursued these issues (i.e. removal of children, foster placements, placement instability, and developmental and behavioral problems) seen in the foster care system and within foster youth through the investigation of a cross fostering model in an animal species. Cross fostering and repeated cross fostering were utilized in a mouse model to explore the impact of this experience of early life stress similar to what is faced by foster youth.

The study examined cross-fostering and repeated cross-fostering as it relates to effects of behavior including learning and memory, activities of daily living, and levels of anxiety. In addition, this study also examined how rearing environment impacts neural development in the brain. To investigate this relationship, behavioral apparatuses and biological tests were utilized to assess behavioral and biological differences. It was hypothesized that mice who were raised in a cross fostering or a repeated cross fostering environment would have increased stress and higher levels of anxiety-like behaviors.

The main objective of this project is to investigate rearing environment and the maternal caretaker (i.e. biological, foster, or repeated foster) and the impact of that early life stress on behavior in an animal model, in order to further inform the scientific literature. A secondary objective is to highlight and disseminate results to those who work with children who have experienced early life stress and/or placement instability and more specifically, children who have gone through or are in the foster care system. The current project seeks to bring attention to the behavioral and neurodevelopmental impact that foster care has on

children and adolescents, especially when compared to those who have not gone through the foster care system.

METHOD

Animals and Housing

Nine timed-pregnant adult ICR (CD-1) female albino mice were purchased timed-pregnant from Envigo RMS Inc. All animals were housed in an Animal Care Systems Optirat semi-self-contained caging system with food and water *ad libitum*. Animals were housed no more than three per cage after weaning. All protocols were approved by the Animal Care and Use Committee of Angelo State University.

Experimental Manipulations

On postnatal day (PND) one, litters were randomly assigned to control, cross-fostering, or repeated cross-fostering conditions.

Cross-fostering (CF). Pups were separated from their biological mother on PND 1 (24 hours after birth) and placed with a foster mother until weaning (PND 21).

Repeated cross-fostering (RCF). Pups were separated from their biological mother on PND 1 and placed with a foster mother for ten days. Pups stayed with their biological mother for 24 hours on PND 1 before being moved to a foster mom. The litters moved from the first foster mom to a second foster mom on PND 11. The litters stayed with the second foster mother until weaning (PND 21).

Control. Pups stayed in the home cage with their biological mother until weaning.

Pups in the control and CF groups were picked up on PND 1 and PND 11 and separated from their mothers and then placed back in their home cages, to control for handling required for the RCF condition. When moving litters, the mother was removed first, then the litter was moved. The new litter in the RCF condition or the same litter in the CF

and control conditions were then placed in the cage and semi-covered with home cage bedding, before the mother is returned to the cage. After PND 11 the litters were left in their home cages and no longer handled until weaning other than weekly cage changes.

PND 1 and PND 11 were chosen as the times for the cross-fostering events because for the RCF group the pups would then be exposed to each foster mother for the same amount of time during rearing before weaning was to occur. Also, Havlicek (2010) found foster youth moved homes on an average of 1.3 times a year. Taking this finding into account and converting human years to mouse days, moving the mice on the first day and then again ten days later is moderately equivalent to the 1.3 times a year in human terms (Dutta & Sengupta, 2016).

Behavioral Measures

Maternal Behavior. After pups were placed in the cage and then the mother was returned, the mother's behavior was observed for 30 minutes. During this timeframe, time spent away from the nest, time spent interacting with the pups (nursing/licking), and time spent nest building were all noted.

Pups were weaned at PND 21 and separated by sex within each litter and moved to separate cages. The pups stayed in this assigned cage for the remainder of the experiment.

Body Weights. Animals in all groups were weighed weekly throughout the project, starting at PND 21.

Open Field Test. The open field test (OFT) is used to assess anxiolytic and exploratory behavior. Mice were gently placed in a 45 cm x 45 cm white box (Harvard Apparatus) and allowed to explore for five minutes for a single trial. Using the SMART

video tracking system (Panlab, Harvard Apparatus) variables including distance traveled, time spent in surround, and time spent in the center were recorded. The behavioral apparatus was thoroughly cleaned with 70% ethanol between animals to reduce olfactory cues.

Elevated Zero Maze. The elevated zero maze (EZM) is used to measure anxiety and risk-taking behavior. The apparatus is an “O” shaped raised platform divided into two sections with walls and two sections with no walls. Mice were placed in the maze facing inward into a closed arm for five minutes for a single trial. The following measures were recorded: time spent in the open arms, time spent in the closed arms, and number of head dips. An animal was considered inside a given region when all four paws were within that area. Head dips were only counted if the animal’s head was over the edge and nose was pointed downward. The behavioral apparatus was thoroughly cleaned with 70% ethanol between animals to reduce olfactory cues.

Morris Water Maze. The Morris water maze (MWM) is used to assesses learning and memory in rodents. The MWM apparatus consists of a 140 cm diameter (Panlab, Harvard Apparatus) tub surrounded by curtains, filled with opaque water. A clear platform was placed in one quadrant just beneath the surface of the water (~ 5-10 mm). Non-toxic white paint was used to make the water opaque and hide the platform. Large cues were placed on the curtains on the outside of the tub in each quadrant. Testing took place over an eight-day paradigm with three trials per day, except for days seven and eight. Animals were habituated to the testing room for at least 10 minutes prior to the beginning of testing. Animals were given 60 seconds per trial to find the platform, with a 45 second intertrial interval (ITI) spent under a heating lamp. On days two, four, and six, the third trial consisted

of a probe trial, where the platform is lowered under the water. Day seven consisted of a single probe trial to assess long-term memory, and day eight consisted of two trials using a visual cue on the platform to assess for visual abnormalities. The animals were tracked using the SMART video tracking system (Panlab, Harvard Apparatus) during this behavioral test. Variables including the following were measured; time spent in target quadrant, latency to find the platform (target), number of target crosses, thigmotaxis, and distance swam.

Activities of Daily Living. The activities of daily living (ADL) tests are used to evaluate normal innate behaviors in the animals.

Burrowing. Burrowing is a natural behavior in mice, used typically for shelter. Burrowing tubes and pea gravel were used to measure burrowing ability of each mouse. Weight of the pea gravel in the burrowing tubes was measured after two and 24 hours.

Nesting. Nesting is important for mice for reproduction and shelter. Nesting was measured using shredded plain white paper. Mice were given 24 hours to build a nest, and nests were scored on a scale of 1 to 5 by rater's blind to experimental conditions.

Biological Measures

Brain Weights. After the animals had been euthanized the brains were removed, weighed, and stored in a -80 °C freezer.

Procedure

Litters were randomly assigned to each experimental group and weaned at PND 21. Animals were tested in two cohorts. The first cohort consisted of control and RCF groups, and the second cohort consisted of control and CF groups.

Maternal behavior was assessed twice, at both cross-fostering events. Body weight was measured weekly starting at weaning (PND 21). Behavioral testing occurred at PND 28 and always occurred during the light phase of the dark-light cycle (8 a.m.- 8 p.m.). The behavioral paradigm was as follows, open field test, one-day break, elevated zero maze, one-day break, Morris water maze, two-day break, and then activities of daily living (burrowing followed by nesting). All experimental animals were euthanized no later than 48 hours after completion of the behavioral testing paradigm.

Maternal Behavior

Mothers were removed from the home cage and placed into another cage. The pups were then transferred between cages in the RCF and CF conditions or picked up, held, and placed back into the same cage (control and CF). The mother was then placed back into the home cage with the pups. The mother's behavior was then observed for 30 minutes. Stopwatches were used for each variable (time spent away from the nest, time spent interacting with the pups (nursing/licking), and time spent nest building). Total time spent in each of these behaviors was noted for each mom at each cross-foster event. Maternal behavior was assessed at PND 1 and PND 11 for each mother.

Love mash (Bio-Serv) rodent reproductive diet (three soft pellets) was added to the home cages on PND 1, PND 11, and PND 16. Addition of love mash to the cages occurred to help with the stress of cross fostering events and to help with pup survival. Love mash was added on PND 16 in cohort one due to the movement of animals to a new lab space, so it was kept the same for cohort two.

Pups were weaned at PND 21. Separated by sex within each condition and moved to new home cages. The pups stayed in this assigned cage for the remainder of the experiment. In cohort one, eight pups were in the control condition from one mom, and 12 pups were in the RCF condition from two moms. In cohort two, 12 pups were in the control condition from two moms, and 16 pups were in the CF condition from three moms. Overall, at the start of behavior, 20 animals were in the control group, 16 in the CF group, and 12 in the RCF group. In cohort one, fifteen pups were lost due to neglect and/or cannibalism. In cohort two, seven animals were lost due to neglect or infanticide, three runts were euthanized at weaning, and seven pups were transferred to another protocol. Tables 1 shows how group numbers changed throughout the experiment and Table 2 lists the animals by group that had been lost or removed.

Table 1

Number of Animals in Each Group

Group	Cohort 1			Cohort 2			Total		
	Birth	PND 21	PND 28	Birth	PND 21	PND 28	Birth	PND 21	PND 28
Control	10	8	8	20	19	12	30	27	20
CF	—	—	—	27	22	16	27	22	16
RCF	24	12	12	—	—	—	24	12	12
Total	—	—	—	—	—	—	81	61	48

Note. Group sizes (n) throughout the experiment.

Table 2

Number of Animals Lost in Each Group

Group	Cohort 1			Cohort 2		
	Death	AP	Euthanized	Death	AP	Euthanized
Control	2	—	—	1	7	—
CF	—	—	—	8	—	3
RCF	12	—	—	—	—	—
Total	—	—	—	—	—	—

Note. AP = Moved to Another Protocol. Number of animals lost or moved in each group throughout the experiment.

Open Field Test

Mice were gently placed in the testing box facing the wall and allowed to explore for five minutes for one trial.

Elevated Zero Maze

Mice were placed in the maze facing inward into a closed arm and allowed to explore for five minutes. All animals were placed in the maze at the same starting point. Time spent in open versus closed arms and number of head dips were recorded. Videos were recorded as data were collected live using stopwatches and a tally counter.

Morris Water Maze

Due to the animals being albino and having a white coat when being placed in white water, animals were marked down their back with a dark colored non-toxic animal marker to allow for tracking of the animals in the camera during the behavioral test. Mice were placed into the tub facing the wall of the quadrant and allowed to swim for one minute. Once the

mouse found the platform, it was allowed to sit for 10 seconds before being taken out. If the mouse did not find the platform within the 60 seconds, the mouse was led to the platform and allowed to sit for 10 seconds. After the third trial the mouse was allowed to stay under the heat lamp until later returned to the home cage. Between each trial the tub was cleaned with a fishing net.

Activities of Daily Living

Both of these behavioral tests were performed with mice placed individually into shoebox cages, with food and water available ad libitum.

Burrowing. Burrowing tubes and pea gravel were used to measure the burrowing ability of each mouse. 2 inch (diameter) PVC pipes were cut to 4.25 inch in length and capped at one end. The tube was filled with 225g of pea gravel. Mice were placed in the shoebox cages by 5:00pm. Weight of the pea gravel in the burrowing tubes was measured after two and 24 hours. The two-hour measure was assessed one hour before the beginning of the dark cycle.

Nesting. Nesting was measured using shredded plain white paper. The paper shreds were cut to be no longer than a few inches in length. 2 grams of paper were weighed for each animal and then sprinkled into each shoebox cage. Mice were given 24 hours to build a nest. After 24 hours, pictures were taken and given to rater's blind to the experimental conditions for scoring of nest building, on a scale of 1 to 5, with 1 being complete scatter of nesting paper to 5 being all the paper was used to construct a nest.

Biological Measures

After euthanasia, brains of all animals were removed and flash frozen. The brains were then weighed and stored in a -80 °C freezer.

RESULTS

Maternal Behavior

Three variables of maternal behavior were assessed at each of the two cross fostering events including time spent away from the nest, time spent interacting with the pups (nursing/licking), and time spent nest building. There was no significance found with any of these variables between the three groups ($p > .05$).

Body Weights

A one-way ANOVA was used to assess weight between groups at weaning (PND 21). This analysis included all animals at weaning ($n = 61$) before some animals were moved to other protocols, others were euthanized at weaning, and three died before behavior began (See Tables 1 and 2). There was a statistically significant difference between groups found, $F(2, 59) = 7.45, p < .01$ (Figure 1). One animal in the RCF group was removed from analysis for being an extreme outlier. Post hoc tests using the Bonferroni correction revealed significant differences in body weight between the groups. Control mice ($M = 10.12, SD = 2.81$) weighed significantly more than the RCF group ($M = 5.84, SD = 1.94$) ($p < .01$). The CF group ($M = 9.90, SD = 5.18$) weighed significantly more than the RCF group ($p < .05$). The RCF group was found to weigh significantly less than both the control and CF groups. The remainder of analysis for body weight assesses the animals at the start of behavior ($n = 48$) (Table 1).

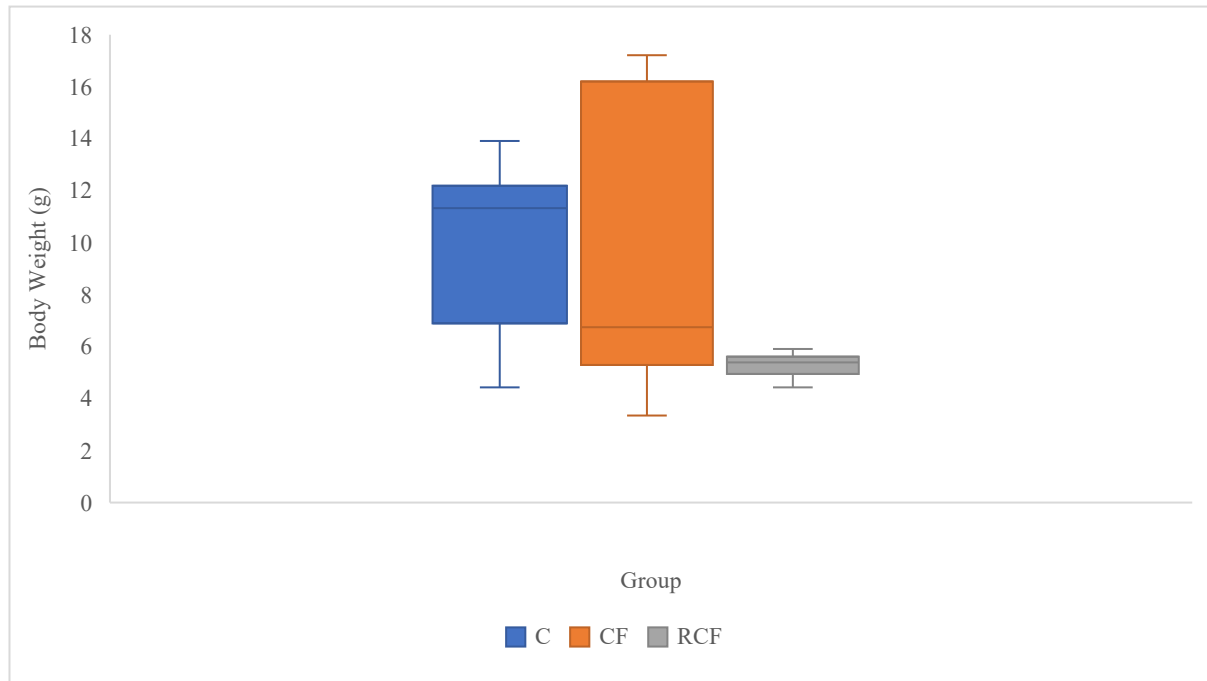


Figure 1. Body weights at weaning (PND 21).

There was a significant change in weight over time in the mice overall found in a repeated measures ANOVA. When comparing general body weight of the foster groups and control groups significant differences were found. During analysis one mouse in the RCF group was removed for being an extreme outlier. Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(9) = 217.89, p < .001$, and therefore, a Greenhouse-Geisser correction was used. There was a significant effect of time on body weight, $F(1.36, 59.76) = 6333.26, p < .001$ (Figure 2). Animals gained weight over time as expected. Post hoc tests using the Bonferroni correction revealed significant differences of body weight between the cross foster and the repeated cross foster groups ($p < .05$). CF mice

($M = 11.51$, $SD = 4.92$) weighed significantly more than the RCF group ($M = 5.84$, $SD = 1.94$)

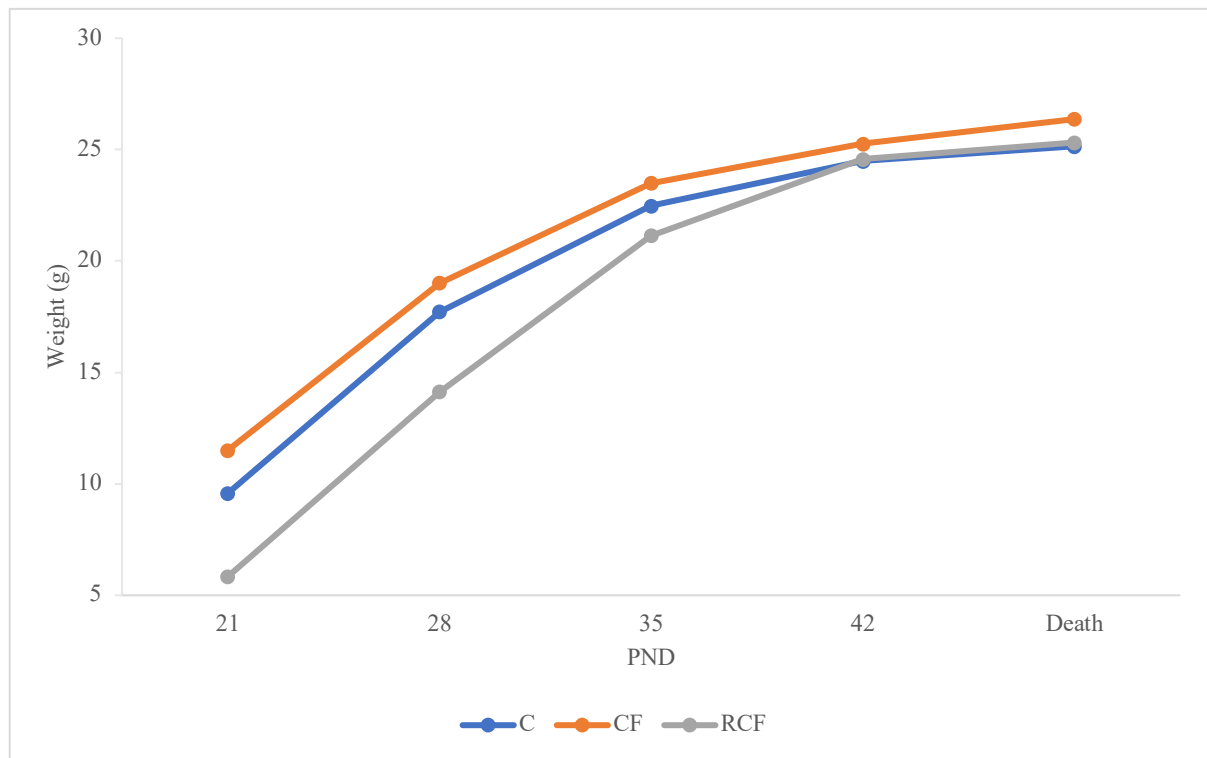


Figure 2. Body weights of animals throughout experiment.

A significant interaction between time and group was also found, $F(2.72, 59.76) = 5.48$, $p < .01$. Post hoc tests using the Bonferroni correction revealed significant differences in weight each week ($p < .001$), as well as for each group separately each week ($p < .001$). A simple effects analysis showed further pairwise comparisons, and significant differences were found between control and RCF ($p < .01$), and between CF and RCF ($p < .001$) in week one. These findings were also found in week two respectively ($p < .05$ and $p < .01$). In week

three, four, and at death there were no significant differences in weight between experimental groups.

Open Field Test

The open field test was used to assess general locomotion and anxiety-like behavior in the mice. When examining percent time spent in the center, percent time spent in the surround, and distance traveled within the apparatus there were no significant differences found between groups using one-way ANOVAs. However, latency to enter the center was trending significant ($p = .07$) between groups. On average, the RCF group was slower to enter the center zone when compared to the control and CF groups (See Table 3).

Table 3

Latency to Enter the Center Zone of OFT

Group	n	<i>M</i>	<i>SD</i>
Control	20	50.84	73.63
CF	16	60.50	86.89
RCF	12	128.27	127.36

Note. Mean and standard deviation of latency in seconds to enter the center zone of the OFT.

Elevated Zero Maze

Anxiety-like behavior and risk-taking behavior was assessed in the mice. One-way ANOVAs were used to assess these behaviors. Time spent in open arms and time spent in closed arms was found to be trending significant ($p = .06$). On average, the RCF group spent

more time in the closed arm when compared to the control and CF groups (See Table 4).

There was a statistically significant difference between groups found for latency to first enter open arm, $F(2, 45) = 8.667, p < .001$ (Figure 3). Four significant outliers in the control group were removed from this analysis. Post hoc tests revealed significant differences were found between control and cross foster ($p < .05$), and between control and repeated cross foster ($p < .001$). Both CF and RCF groups took longer to first enter an open arm when compared to the control group. (See Figure 3).

Table 4

Time Spent in the EZM

Group	n	Open Arm		Closed Arm	
		<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
Control	20	6.75	6.78	93.25	6.78
CF	16	4.67	6.41	95.33	6.41
RCF	12	1.50	2.57	98.50	2.57

Note. Mean and standard deviation of percent time spent in open and closed arms of the EZM.

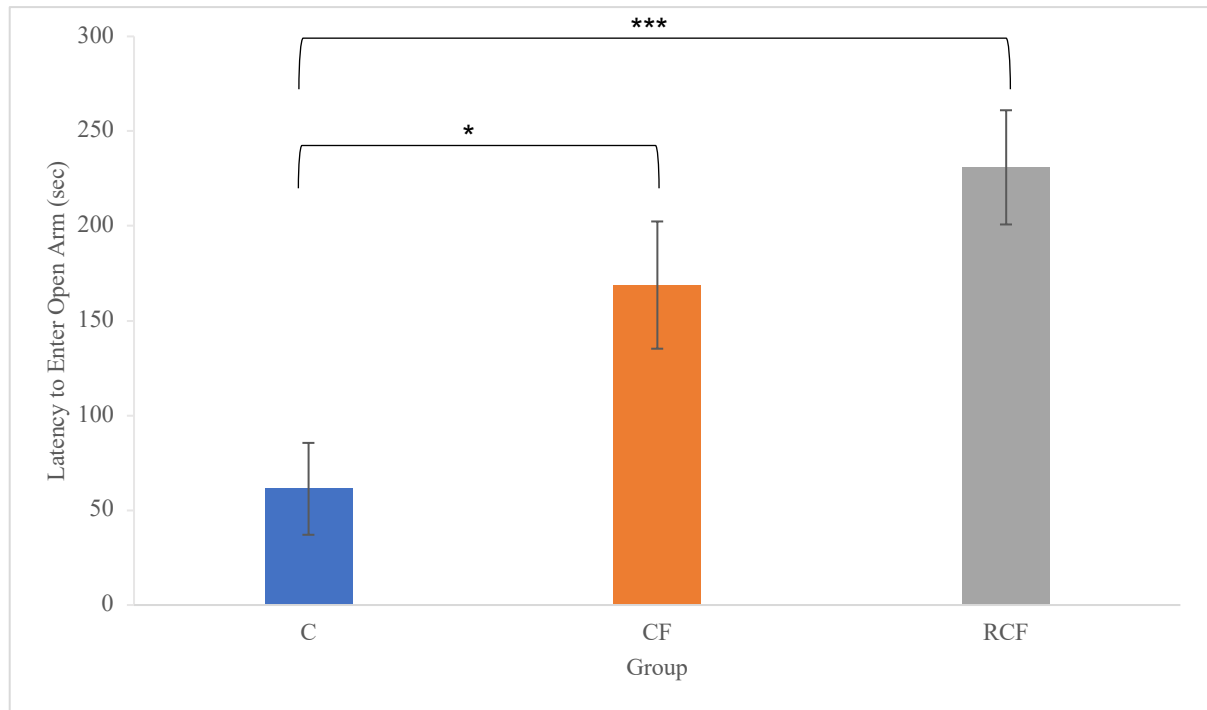


Figure 3. Latency to enter the open arm of the EZM.

* $p < .05$ ** $p < .01$ *** $p < .001$

There was also a significant difference between groups for head dips, $F(2, 45) = 5.219, p < .01$ (Figure 4). A post hoc test revealed a statistically significant difference between control and repeated cross foster mice ($p < .05$), and cross fostered and repeated cross foster mice ($p < .05$) (See Figure 4). Controls were found to have more head dips when compared to repeated cross fostered mice, and cross fostered mice were found to have more head dips as compared to repeated cross fostered mice.

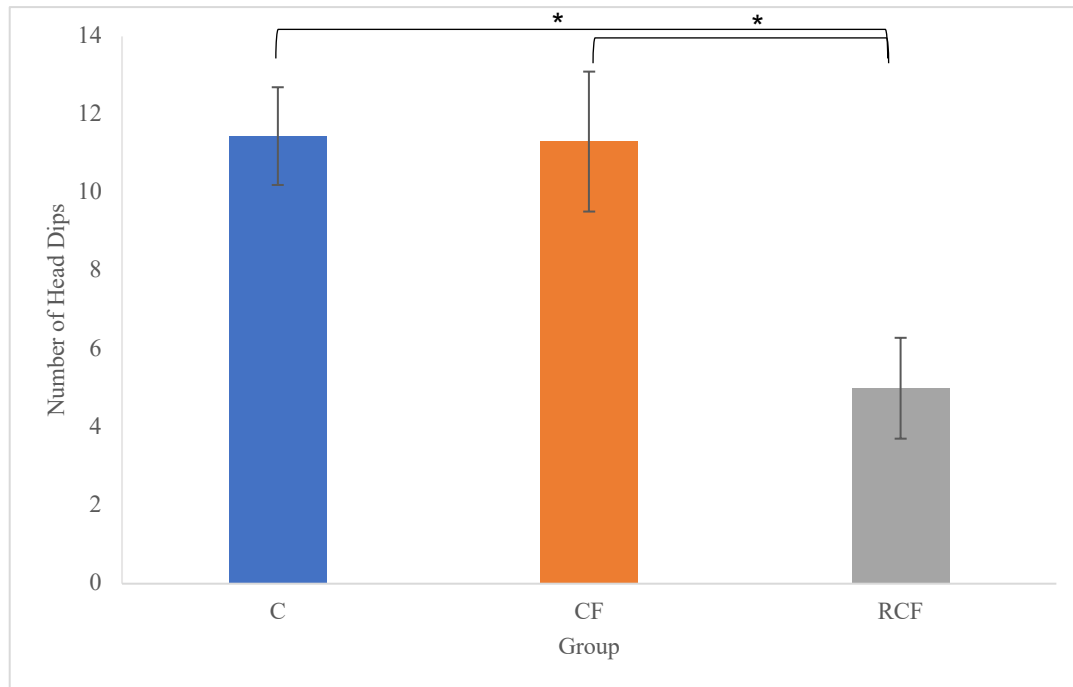


Figure 4. Head dips during the EZM.

* $p < .05$ ** $p < .01$ *** $p < .001$

Morris Water Maze

The MWM was used to assess both short-term and long-term memory in the mice. Repeated measures ANOVAs were used to assess variables in the Morris water maze. There was a significant effect of day for latency of the animal to reach the platform, $F(5, 220) = 3.987, p < .01$ (Figure 5). One outlier in the RCF condition was removed when assessing latency. A post hoc test revealed a statistically significant difference between day 1 and day 6 ($p < .05$).

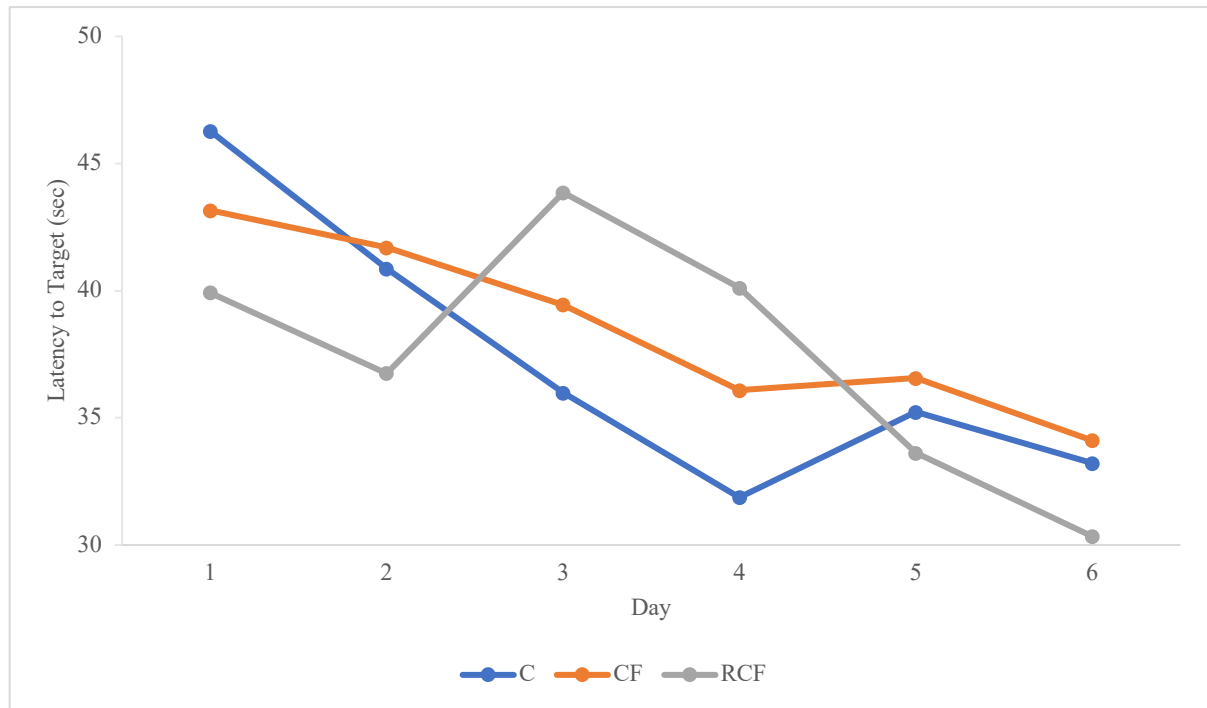


Figure 5. Latency to target in the MWM.

Thigmotaxis, the percent time spent along the outside border of the maze, was assessed for each animal. Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(14) = 31.23, p < .01$, and therefore, a Greenhouse-Geisser correction was used. Four animals were removed from this analysis due to being significant outliers. There was a significant effect of day on time spent in the border of the pool, $F(3.96, 162.51) = 46.76, p < .001$ (Figure 6). A post hoc test revealed a statistically significant difference between day 1 and days 3 - 6 ($p < .001$). As days progressed, thigmotaxis decreased.

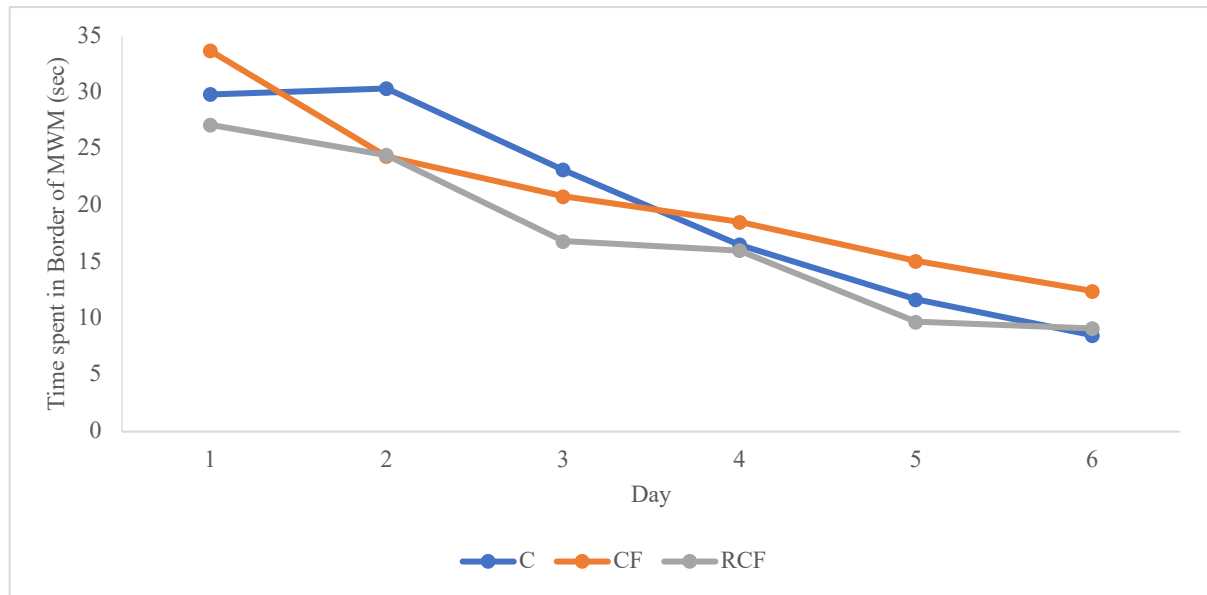


Figure 6. Thigmotaxis in the MWM.

Time spent in the target quadrant was also assessed. Significance of day for time spent in target quadrant was trending ($p = .056$). (See Table 5). There was also a significance found for effect of day on distance traveled in the tub $F(5, 225) = 5.184, p < .001$ (Figure 7). A post hoc test revealed a statistically significant difference between day 2 and day 5 ($p < .001$), and between day 4 and day 5 ($p < .05$). As days passed mice traveled a shorter distance in the tub overall.

Table 5

Time Spent in Target Quadrant of MWM

		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Group	n	<i>M (SD)</i>	<i>M (SD)</i>	<i>M (SD)</i>	<i>M (SD)</i>	<i>M (SD)</i>	<i>M (SD)</i>
Control	20	23.05(6.19)	23.81(3.08)	22.39(3.80)	22.54(5.40)	21.67(7.43)	23.98(7.41)
CF	16	20.80(5.96)	20.71(5.86)	22.29(5.36)	24.16(4.82)	22.78(5.78)	24.95(5.35)
RCF	12	23.40(5.83)	23.55(9.78)	19.35(5.53)	17.91(4.02)	18.53(6.57)	24.52(7.14)

Note. Mean and standard deviation of time spent in target quadrant of the MWM.

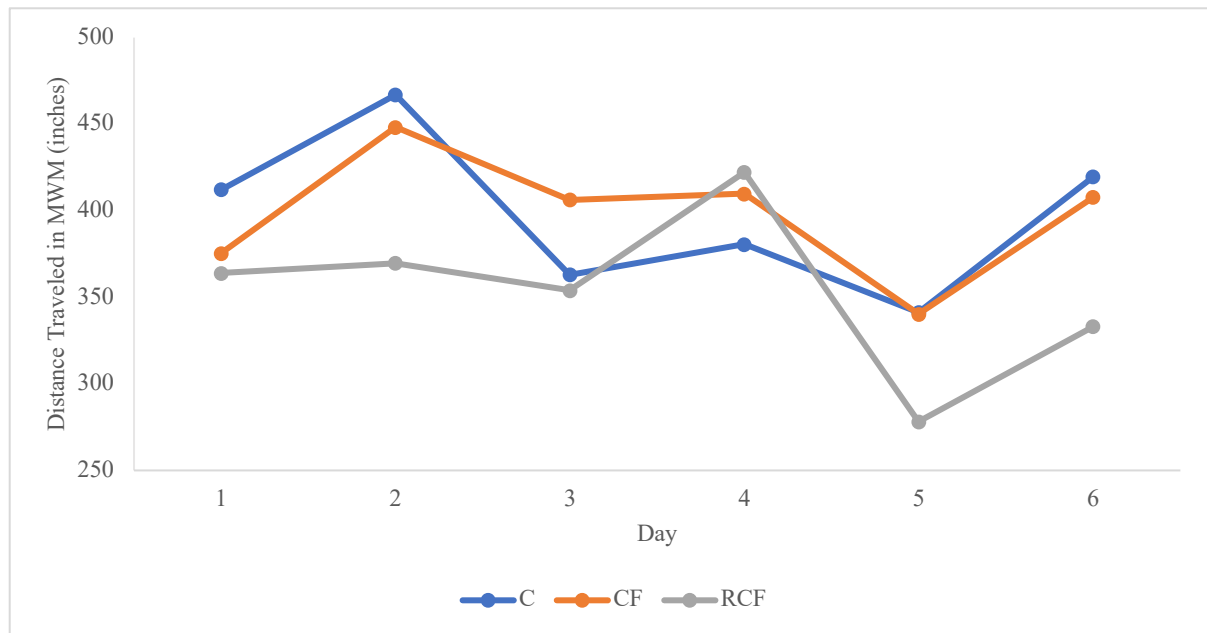


Figure 7. Distance swam in the MWM.

When assessing crosses into the platform zone (target) on probe days (days 2, 4 and 6) there was no significance found. There was also no significance found with latency to target or number of target crossings on day 7.

Activities of Daily Living

Innate behaviors of daily living were assessed in these mice, by investigating both burrowing and nesting.

Burrowing. There was no significance found in burrowing between the groups at either the two hour or the 24-hour time mark.

Nesting. Raters blind to the conditions rated nesting on a score of 1 to 5. With a score of 1 being a complete scatter of nesting paper to a score of 5 when a mouse has used all of the paper in the cage to build a nest. Interrater reliability was found to have a Cronbach's alpha of .82. Using a one-way ANOVA, there was a significant difference between groups in nest building behavior, $F(2, 45) = 4.439, p < .05$. Post hoc analysis revealed a significant difference between the cross foster ($M = 4.13, SD = 0.63$) and repeated cross foster ($M = 4.77, SD = 0.31$) groups, with the repeated cross fostered animals building better nests overall when compared to the cross fostered group ($p < .05$). Figure 8 shows representative nests built by animals in each group.

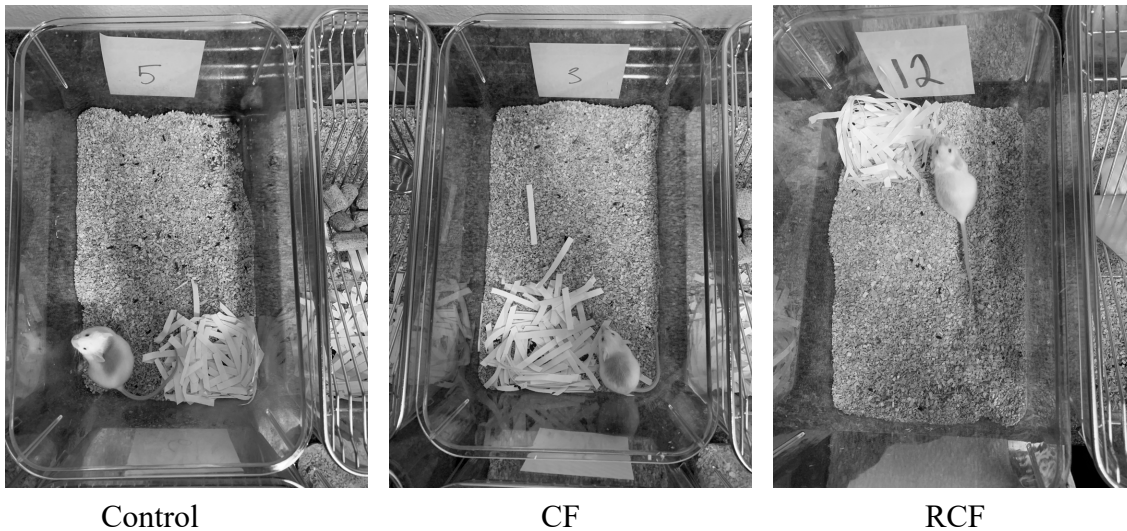


Figure 8. Representatives of nests built in each group.

Biological Measures

Brain weights were gathered for foster groups and control groups. There were significant differences in brain weight between the control group, CF group, and the RCF group $F(2, 45) = 8.759, p < .001$ using a one-way ANOVA (Figure 9). Post hoc tests revealed significant differences between control and cross foster groups ($p < .05$), as well as between cross foster and repeated cross foster groups ($p < .001$). Weights of brains for the cross-foster group were found to be heavier than those in the control group, as well as heavier than those in the repeated cross-foster group.

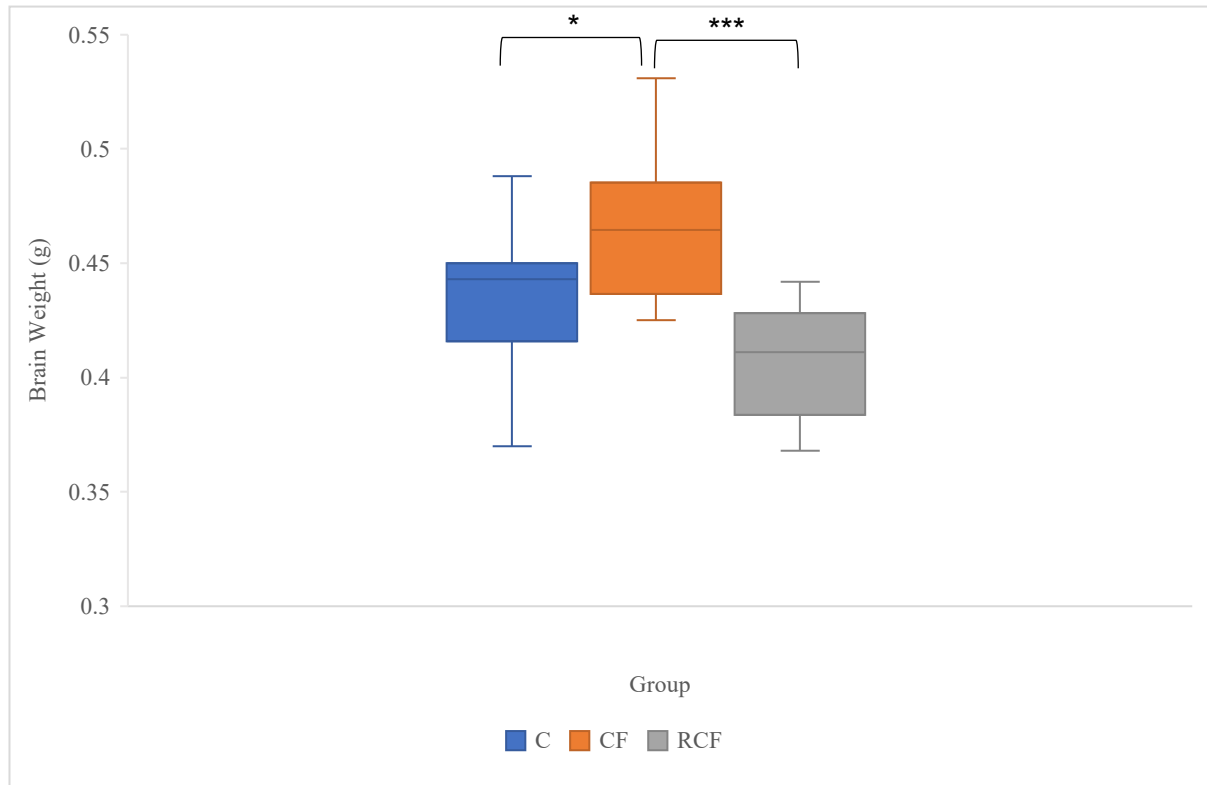


Figure 9. Brain weights of animals.

*p < .05 **p < .01 ***p < .001

Criteria for removing outliers for all of the above was the data point being more than three standard deviations away from the mean, and removing the outlier allowed the data to no longer violate normality. All data were analyzed using SPSS Statistics software.

DISCUSSION

The aim of this study was to investigate rearing environment and early life stress in three different postnatal manipulations specifically in ICR (CD-1) mice. These manipulations included control mice (stayed with biological mother), cross foster mice (moved to a foster mom on PND 1), and repeated cross foster (moved to foster mom one on PND 1 and then to foster mom two on PND 11). Mice in fostering groups did exhibit anxiogenic behavior, thus supporting the hypothesis. The few studies investigating cross fostering and repeated cross fostering were specifically examining a certain behavior or examining long-term impacts of CF and/or RCF. In this project short-term impacts on many behaviors including cognitive and non-cognitive behaviors were assessed.

Behavioral testing started at PND 28 which is the end of adolescence for mice. This is equivalent to about 12 years old in human age (Dutta & Sengupta, 2015). The goal of this project was to assess early life stress and its impact on behavior of the offspring in the short-term. To then be able to bring to light more information about the impacts that occur to offspring, whether that be mouse or human, when stress and environment changes were experienced in early adolescence.

In this project, by investigating cross-fostering and repeated cross-fostering it can be examined how the type of rearing environment impacts behavior and neurological changes in an animal model. This design can then be implicative of what foster children experience when they are moved from one home to another and raised by a nonbiological parent.

The current study demonstrates how being raised by a foster mother when compared to being raised by a biological mother significantly affects emotionality and development in

the offspring. This impact is especially seen when offspring are raised by multiple foster mothers and moving environments multiple times during childhood. These results can be related back to what children in the foster care system experience in early life and how that experience impacts them in physical and mental development and health. This study brings to light the importance of early life experiences and how the experience of stress early in life, specifically placement changes and changes in rearing caretakers, can have a large impact on emotional and developmental trajectories and overall health.

Body Weight

Specifically, at weaning RCF mice weighed significantly less than mice in the control group and also weighed significantly less than mice in the CF group. Overall, animals gained weight overtime which is expected with growth and aging. Mice in the RCF group weighed significantly less than those in the control group between weaning and at the end of the experiment. More specifically, it was shown that animals in the RCF group weighed significantly less than those in the control group as well as those in the CF group in weeks one and two.

This result suggests that the more extreme the manipulation the more impact is seen with weight, from the beginning at weaning and throughout the time of testing. This finding shows how rearing environment can play a role in the development of the mice and how that deficit in development can continue over time.

Elevated Zero Maze

Both CF and RCF groups took longer to enter the open arm once placed in the EZM when compared to the control group. The latency to enter the open arm is a measure of

anxiety-like behavior (Braun et al., 2011). These results suggest that the CF and RCF mice have significantly more anxiety-like behavior than do the control mice.

Head dips are also another index of anxiety-like behavior, with animals who are exhibiting more anxiolytic behavior having a higher amount of head dips during the EZM trial (Takeda et al., 1998). Head dips are also a measure of risk-taking behavior in rodents (Walf & Frye, 2007). RCF mice were found to have significantly fewer head dips when compared to the CF mice and the control mice. This suggests the RCF mice were expressing higher levels of anxiogenic behavior and were less likely to take a risk when compared to both the CF and/or the control mice.

Overall, CF and RCF mice were found to have higher levels of anxiogenic behavior which is consistent with findings of experience of early life stress in mice, in that mice who have experienced early life stress have been found to have more anxiogenic behaviors and reduced emotionality (Lerch et al., 2014; Luchetti et al., 2015). Literature shows similarities in humans and specifically in foster youth (Morton, 2017). Foster youth are a population more at risk for mental health challenges and have been found to experience more anxiety when compared to children not in the foster care system (Leve et al., 2013; Morton, 2017).

Morris Water Maze

In this learning and memory behavioral test, no differences between groups were found. Overall, mice were able to learn over time, by finding the platform faster and swimming a shorter distance over the six days. Mice were also showing less thigmotactic behavior overtime with decreased time spent in the border of the pool against the wall.

The cross-fostering model used did not have a significant impact on learning and memory as has been shown in other animal studies with the Y-maze and novel object recognition (Lu et al., 2009). Further analysis is needed here in the investigation of the impact on memory due to the studies showing the vast changes in brain development and memory we see in humans who experience foster care and early life stress.

Activities of Daily Living

There were no findings within the burrowing assessment, suggesting that early life stress does not play a role in this specific non-cognitive task. However, mice who had been exposed to multiple foster mothers in the RCF condition were able to build significantly better nests than those in the CF condition. This ability to build better nests could be a sign of resiliency in the mice, with those who experienced higher amounts of early life stress and placement moves, relying more on their innate behaviors because they did not have the same consistency of maternal care and a maternal bond as did the mice in the other conditions.

Resilience is a dynamic interplay of personal and environmental factors (Hass et al., 2014). In the few studies that exist it has been shown that resilience is not a common phenomenon in foster youth (Hines et al., 2005). Due to this knowledge it could be that foster youth are not as able to adapt and perform non-cognitive behaviors as well as their non-foster peers. Indeed, results show a resilience in the RCF mice, which could also potentially be seen in foster youth, but foster youth often don't have supportive systems to help build this resilience. Support systems, especially with a non-abusive adult is crucial in promoting resilience in maltreated children (Hass et al., 2004; Hines et al., 2005).

Another explanation could be that the mice in the RCF group are relying more on their innate behaviors because the early environment was unstable, and due to the movements, the maternal bond was not as strong, leading to a reliance on the behaviors that they do know. Foster youth have been shown to be more self-reliant and independent sooner when compared to other youth, this could also be what is being seen in this non-cognitive behavior (Steenbakkens et al., 2018).

Biological Measures

The brains of mice in the CF group weighed significantly more than brain weights in the control group and weighed significantly more when compared to mice in the RCF group. Brain weight or brain volume can be considered a measure of cognitive or brain reserve (Murray et al., 2011). This measure of greater cognitive reserve can then help protect one against the destructive effects of neuropathology (Murray et al., 2011). With the RCF group having smaller volume of brain matter and therefore less cognitive reserve when compared to the CF group can suggest how going through extenuated experiences of early life stress can impact the development of the brain. It is possible that the repeated stressors of changing mothers' multiple times during development played a larger role in the developing brain than did the movement once very early in life.

The CF group also had larger brains than the control group. This difference could be due to a form of resiliency showing up in the group that experienced an early life stressor. It is possible that the chronic stressor experienced by the RCF group negated the resiliency in this cognitive manner, whereas the CF group were able to respond to their environment in a superior way due to the manipulation not being as severe. Chronic stress leads to reductions

in neuronal volume while acute stress does not (Bremner, 2006). This could explain why RCF mice have a reduction in brain volume while CF mice do not. Specifically, early life stress can lead to enlargements in stress-sensitive areas of the brain (Spinelli et al., 2009). This concept can help explain as to why the CF group has larger brain volumes when compared to the control group.

Limitations

There were a few limitations worthy of note. First, the first cohort of animals were moved to a new lab space in the middle of the experiment. The cross fostering events took place in two different spaces for the two cohorts, and five days after the RCF group was moved to their second foster mother, they were then moved across our campus and into a new space. Steps were taken to ensure the transition was as smooth as possible by moving mice across campus during non-peak hours. This move could have caused undue stress on the first cohort, in which the second cohort did not experience.

One limitation was the method of collecting data for maternal behavior. There was no interrater reliability reported for this measure due to only one researcher collecting data for each mother. Keeping track of multiple behaviors by one person could have limited the accuracy of the data collected if multiple behaviors were happening at one time point.

To note, as opposed to other models of cross fostering, due to limited lab space, animals only experienced cross fostering events twice throughout infancy and childhood versus four times in the four days directly after birth.

Future Directions

In the future, brain specimens from this project will be utilized to investigate

biological measures such as stress hormones and/or BDNF presence in the brain.

Investigating the brains of the mice will allow for information on how this type of manipulation and rearing environment impact the neurochemistry of the brain. This information could then be compared to the changes seen in human brains and in human brain development of children who have experienced early life stress. Future research could also investigate implementing a larger manipulation in the CF and RCF groups to see if then there would be learning and memory differences between the groups. Also examining the short-term and long-term impacts of this type of manipulation is important to see if the differences found in this study could also be seen in the long-term.

CONCLUSION

The maternal environment and early life environment play a critical role and have significant impacts on the behavior of offspring. Results show how the environment impacts body weight, brain weight, anxiety-like behavior, risk-taking behavior, and the non-cognitive behavior of nest building. All of these factors need to be considered when thinking about children in the foster care system and how the experiences these children are going through have the ability to impact so many aspects of their health, development, and general life.

Cross fostered mice showed more resiliency in cognition, while RCF mice showed more resiliency in non-cognitive behavior. Resiliency is a positive adaptation towards adversity (Hass et al., 2014). This topic is a gap in research that needs to be explored more in foster youth, and how they are or are not able to adapt and overcome.

Funding

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APPENDIX

IACUC APPROVAL



ANGELO STATE UNIVERSITY

College of Graduate Studies & Research

Institutional Animal Care & Use Committee

05/21/19

Crystal Krietler, Ph.D.
Associate Professor
Director, MS Program in Experimental Psychology
Department of Psychology & Sociology
Angelo State University
ASU Station #10888
San Angelo, TX 76909

Dear Dr. Krietler:

Your proposed project titled, "The Examination of Cross-Fostering with NMRI Mice as a Model for Foster Care" was reviewed by Angelo State University's Institutional Animal Care and Use Committee (IACUC) in accordance with the regulations set forth in the Animal Welfare Act and P.L. 99-158.

This protocol was approved for three years, effective 05/21/19, and it expires three years from this date; however, an annual review and progress report form (www.angelo.edu/content/files/22583-iacuc-annual-review-progressreport) for this project is due on May 19th of each year. If the study will continue beyond three years, you must submit a request for continuation before the current protocol expires.

The protocol number for your approved project is 19-205. Please include this number in the subject line of in all future communications with the IACUC regarding the protocol.

Best,

Steven



Steven T. Brewer, PhD
*Assistant Professor,
Director, MS Program in Experimental Psychology
Co-Chair, Institutional Animal Care and Use Committee
Psychology & Sociology
Angelo State University
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BIOGRAPHY

Marisa Diane Thompson finished her Bachelor of Science degree at Angelo State University in May 2018. She graduated cum laude with High University Honors with a double major in Biology and Psychology, and a minor in Chemistry. She will be graduating with her Master's of Science in Experimental Psychology (with an emphasis in behavioral neuroscience) in May of 2020. Marisa was highly involved in research during her masters, completing many projects in both human and non-human animal models, and presenting at both regional and international conferences. She also started a student organization for graduate students involved in research, to help build a community. Marisa will continue with her passion for research and psychology in her career.

Marisa Thompson can be contacted at marisathompson95@gmail.com